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14. ABSTRACT

The goal of this project is to record activity of serotonin and dopamine neurons during goal directed behavior. During this project we have overcome number of technical hurdles in order to successfully record neural activity of neurons within the raphe nuclei of behaving rats. Contrary to earlier studies that reported mainly tonic long timescale changes in firing rates, we found that raphe neurons phasically respond to task events on a short timescale of milliseconds during various phases of the task spanning sensory, motor and reward related processes. We are encouraged by these results and are confident that this approach of recording from raphe neurons during goal directed behaviors will provide a new understanding on function of serotonin in aspects of cognition, emotion and behavior. We now plan to investigate raphe neuronal responses during specific behaviors requiring serotonin function such as delayed reinforcement and reversal learning. We also plan to study interaction between raphe neuronal firing and sniffing and other motor behaviors. Having established the technically challenging raphe recording technique we can now perform dual serotonin and dopamine neuron recordings during goal directed behavior. Together, these studies are likely to provide insights into how disruption of normal Da/5HT function leads to motor and motivational impairments seen in Parkinson's Disease.

15. SUBJECT TERMS
Parkinson's Disease, serotonin, dopamine, raphe, reward learning

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INTRODUCTION

The goal of this project is to record neural activity of serotonin (5HT) and dopamine (DA) neurons in rats during the performance of a reinforcement learning task. We are interested in how neural activity within these ascending modulatory systems is temporally related to events during learning and decision making. Electrophysiological studies of ventral tegmental area (VTA) neurons in monkeys by Wolfram Schultz's group have led to a breakthrough in understanding of the DA system by showing that their phasic firing patterns correspond to an important variable posited by reinforcement learning theory, the reward error (or temporal difference) signal. These studies have not yet been replicated in rats. In doing so, the ongoing work will provide a window onto the function of the DA system that is complementary to the rich existing knowledge from pharmacological and biochemical assays. Even less is known about the relationship between dorsal raphe (DR) 5HT neuron firing and decision variables in any species. Indeed, there are no studies characterizing the behavior of 5HT neurons during the performance of a behavioral task in any species. The major goal of the ongoing work is to identify phasic behavioral correlates of 5HT neural activity. Over the course of this project, we have overcome many of the technical hurdles associated with recording from raphe neurons in awake behaving animals. We have found that raphe neurons show phasic modulation of firing rates to the order of milliseconds in response to specific task events. Thus, we have made significant strides towards our goal of characterizing neural activity within the serotonin and dopamine systems during goal directed behavior.

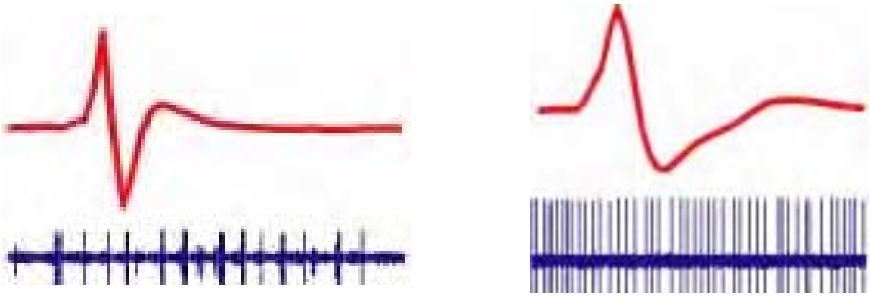
RESULTS

Anesthetized Experiments

Localizing and Identifying Single Cells within the Dorsal Raphe Nucleus

Forebrain projecting serotonin neurons are principally located within two midline nuclei – the dorsal raphe and median raphe nuclei. Because these nuclei

Figure 1: Neuronal cell types from dorsal raphe nucleus of anesthetized rats.



Left panel : wide action potential waveform of a putative 5HT neuron and spike train exhibiting its regular firing pattern. *Right panel* : narrow action potential waveform of a fast firing non-5HT neuron and an example trace showing bursty, irregular firing

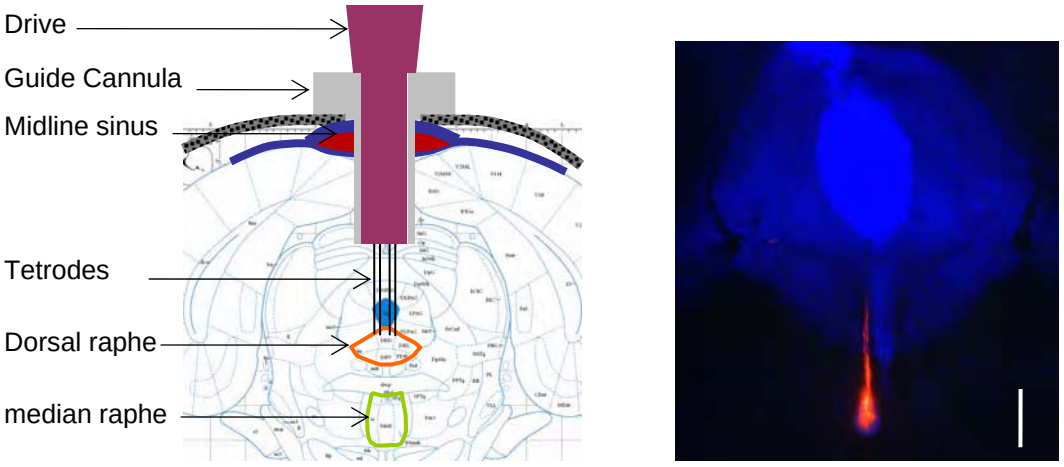
contain a variety of cell types in addition to serotonin neurons, it was important to establish our ability to reliably identify distinct cell classes within the raphe nucleus using standard electrophysiological criteria prior to the initiation of awake recordings. Serotonin neurons are identified by their wide action potential, low spontaneous firing rate and regular firing pattern. In addition serotonin neurons are inhibited by the 5HT1a receptor agonist. We carried out a series of recordings using sharp-tungsten electrodes in the anesthetized rat. Consistent with previous studies, we find at least two distinct cell types, examples of which are shown in Figure 1. The first cell class consists of putative GABAergic interneurons characterized by their narrow waveform and high firing rate (Fig.1 *left panel*). The second cell class consisting of cells with wide action potentials and spontaneous firing rates of around 0.5-2 Hz is characteristic of serotonin producing neurons (Fig. 1, *right panel*). Pharmacological experiments are underway, however histological localization of our electrodes and other firing characteristics provide evidence that these are putative serotonin neurons.

Awake Experiments

Technical hurdles and solutions for raphe recordings in awake rats

Since we are especially interested in characterizing interactions between cells within the raphe, we decided to use tetrode (four channel) recording electrodes in favor of single wires for awake experiments. By using a process

Figure 2: Novel approach for raphe recordings in awake rats using tetrodes.



Left panel: Schematic diagram of guide cannula and tetrode drive implantation into dorsal raphe nucleus. *Right panel*: DiI track created by tetrode penetration visualized by fluorescence microscopy clearly shows track passing through dorsal raphe (DR). Scale bar: 0.5 mm

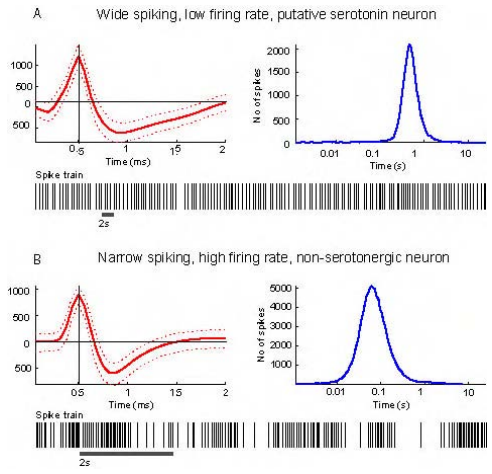
analogous to triangulation, tetrodes are capable of segregating signal sources (i.e. individual neurons) thereby allowing simultaneous recording of several neurons. However, in the process of switching from tungsten electrodes to tetrodes a number of technical hurdles had to be overcome. In particular, the anatomical location of the raphe nuclei is such that the most direct electrode

penetration passes thru both the midline sinus (plexus of blood vessels) and the cerebral aqueduct. While sharp electrodes are able to puncture the sinus and the thick dura below it, tetrodes are not stiff enough to pass through. While it is possible to bypass the sinus by penetrating laterally at an angle, it is very difficult for reliable stereotactic targeting since the raphe nucleus is a small structure. It also limits our ability to record from both the dorsal and medial raphe nucleus on a single electrode penetration. As a consequence, we developed a novel two-step approach for tetrode targeting and implantation. In the first part of the procedure, a hole is drilled in the skull on the midline above the raphe (-7.5 mm A.P. relative to bregma) and a stainless steel guide cannula (18.5 gauge) with a stylet to maintain patency is lowered into the brain. The cannula tip is at a depth of ~ 3.0 – 3.5 mm from the skull surface (~ 2mm above the raphe). Tetrodes are lowered into the brain through the cannula. Using this procedure we are able to stereotactically localize tetrodes into the raphe nucleus as demonstrated by fluorescent labeling of tetrode tracks stained with DiI (Fig. 2).

Raphe Recordings in the Awake Rat: Neuronal cell types

Using the above mentioned method we successfully recorded neural activity from DR nucleus of awake freely behaving rats. Chronically implanted

Figure 3: Neuronal cell types from DR nucleus of awake rats



Firing properties of putative 5HT neuron (A) and non-5HT neuron (B) For each neuronal cell type, typical action potential waveform (left, red trace), Log isi distribution (right, blue trace) and representative spike train (bottom) are shown. Scale bar: 2 sec

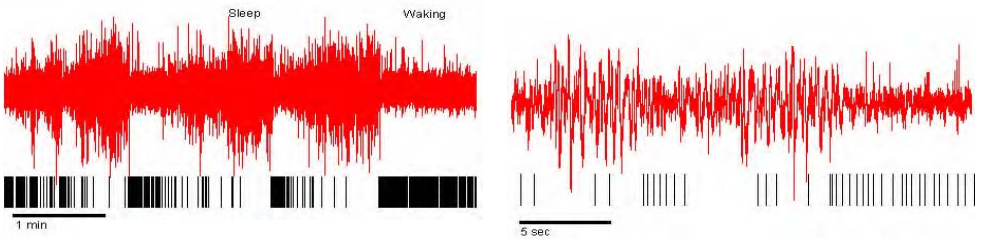
animals recovered completely from surgery, were healthy and showed no gross behavioral deficits. Their performance on 2 odor discrimination task was identical to that before surgery. Putative 5HT neurons were identified on the basis of their wide action potential and slow regular firing pattern (Fig. 3A). Non-5HT neurons had a narrow spike and showed variation in their firing rates. Fig. 3B shows an example of a narrow spiking non-5HT neuron with high spontaneous firing rate and irregular firing pattern. A small subset of narrow spiking neurons also had a

slow spontaneous firing rate. Thus, we are able to record from raphe neurons in awake rats using tetrodes.

Tonic changes in firing rate across sleep wake cycle

Previous studies in cats and rats have demonstrated firing rate changes in serotonin neurons across the sleep – wake cycle. In our recordings we have also found neurons that exhibit these properties. An example neuron is shown in Fig. 4. This is the same neuron shown in Fig. 3A.

Figure 4: Tonic changes in firing rate of putative 5HT neuron across sleep wake cycle



Red traces are hippocampal EEG recordings and *black* ticks are spikes aligned to EEG trace during two epochs showing marked inhibition of firing during large amplitude activity associated with slow wave sleep (SWS) and increased firing at transitions from SWS to wake. Scale bars: left: 1min and right: 5 sec

This neuron shows a marked suppression in firing as the animal transitions from waking to slow wave sleep (SWS). SWS is characterized by large amplitude activity in the 1-5 Hz in the hippocampal EEG [1]. While the tonic firing rate does not change much over the course of a behavioral epoch, the neuron increases its firing rate transiently at the beginning of a sleep to waking transition. Firing rate changes with behavioral state have been reported for serotonin neurons [2], however, there is no evidence in literature for increased firing at state transitions.

Raphe recordings during goal directed behavior

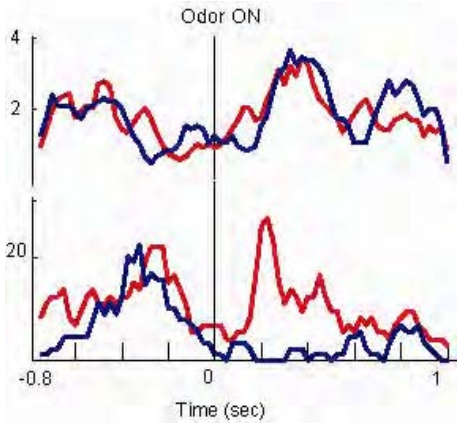
Behavioral correlates of raphe neuronal firing – Phasic modulation

Extensive pharmacological and lesion studies suggest a role for forebrain serotonin in reward learning as well as impulsivity [3]. However, there are no studies of raphe neuronal responses during behavior. Thus our understanding of serotonin function in the brain is limited to long term effects on animal behavior due to inherent large time constants of action for drugs and lesions (several minutes to days). Raphe recordings from awake animals also focus on elucidating tonic firing rate changes. Thus there is no data available on response properties of raphe neurons during goal directed behavior. This approach has been especially successful in the study of another neuromodulatory system namely dopamine [4]. Therefore one of the major goals of this project was to record from raphe neurons during goal directed behavior and correlate phasic modulation of firing rate with specific behavioral events over short timescales of milliseconds. While serotonin neurons are responsible for 5HT release in target structures, their firing and therefore release is regulated by non-5HT neurons in

raphe. (e.g. GABAergic interneurons). Therefore we were interested in recording from both 5HT and non 5HT neurons. Over the past several months we have obtained several recordings from neurons in the DR while the rat is engaged in a 2 odor discrimination task.
Water deprived rats were trained to poke into a center port where on a given trial one of two odors was delivered. Each odor was associated with availability of water at one of two choice ports (Left and Right). Depending upon odor identity the rat learnt to choose the correct port to receive water reward. Rats performed this task very well with more than 90% correct responses[5]. There was no change in performance after drive implantation surgery. Timing of all task events was recorded with millisecond precision enabling us to align spikes to various behavioral events and investigate phasic neuronal responses. With this task we can study correlations in firing of raphe neurons during various aspects of behavior including sensory, motor, decision and reward processes.

Modulation during odor sampling:
Raphe neurons heavily project to the MOB as well as other olfactory areas. Activity of subset of serotonin neurons increases during sniffing [6]. Similarly, Barry Jacobs [7] postulated the motor hypothesis outlining a model in which activation of serotonin neurons facilitates motor output while simultaneously inhibiting sensory processing. Thus we expected to see modulation of neuronal firing during odor sampling phase of the task.
As expected, 33% of neurons recorded showed odor sampling related modulation. There were examples of both activation and inhibition (Fig. 5). Top

Figure 5: Firing rate modulation during odor sampling.



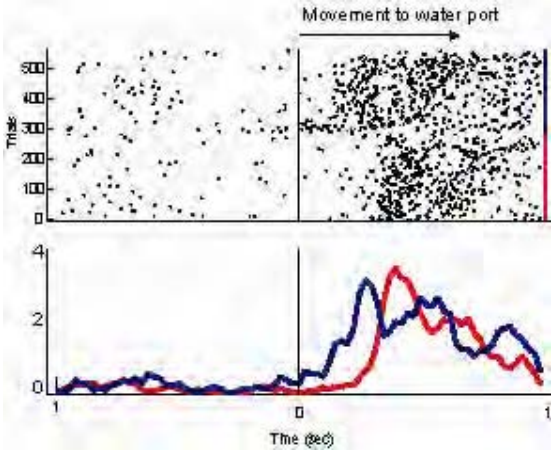
Top panel: Peri-stimulus Time Histogram (PSTH) of putative 5HT neuron showing inhibition during odor sampling. Spikes are locked to time of Odor onset and averaged over trials. *Bottom panel*: PSTH of a non-5HT neuron showing activation to 1 odor from the pair. *Red* and *blue* traces denote trials with the 2 odors.

trace is of a putative 5HT neuron same as in Fig. 3a and 4. This neuron was inhibited during odor sampling phase consistent with prediction of the motor hypothesis. However, neuron in the bottom trace (a narrow spiking, high firing rate, non-5HT neuron) showed odor specific activation only to 1 odor from the pair. While this neuron could be a non-5HT GABAergic interneuron that might

inhibit the 5HT neuron, the stimulus specificity of the activation is hard to explain. This specificity may be imparted by other higher order differences between the 2 stimulus reward contingencies.

Modulation during movement:
Serotonin has been hypothesized to play an important role in central control of motor function. Raphe neurons change their firing rate during treadmill induced locomotion as well as other motor activities such as grooming and other central pattern generator (CPG) mediated behaviors [6]. Consistent with this expectation, Figure 6 shows a neuron that increased its firing rate during movement from the center port to the water port on both sides. However, this increase was not observed while approaching odor port or going away from water port. This leads us to speculate that this neuron might be modulating a stereotyped motor pattern since all other movements are not as stereotyped as movement from center port to water port(s). However, the specificity may even be correlated to reward expectation. These possibilities can be teased out by comparing trials on which animal expected a reward to trials where the animal did the same movement but did not expect a reward by employing a fixed ratio

Figure 6: Modulation of raphe firing during movement.



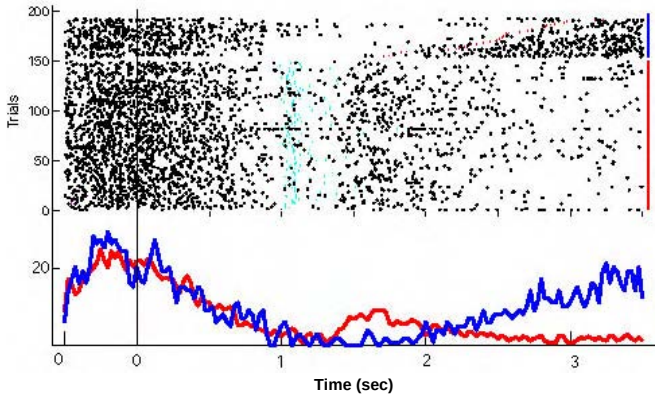
Top panel shows a raster plot of a non-5HT neuron locked to exit from the odor port. *Bottom panel* shows PSTH of the same neuron. *Blue* and *red* traces are for movement to left and right sides respectively. Firing rate increases as the animal approaches the water port. Entry into water port is denoted by blue dots. Trials are sorted by increasing movement time.

reward schedule. We would also like to test the effect of increased effort on such responses since serotonin has been implicated in such behaviors [8].

Activity during reward delay:
Serotonin is known to be involved in behaviors related to time discounting such as inter-temporal choice behavior, temporal discounting, delayed reinforcement as well as impulsive choice behavior through pharmacological and lesion studies [9]. However how this relates to changes in firing rate of raphe neurons is not known. We expect raphe neuronal activity to be strongly influenced by delay to reward. To study this aspect of serotonin function, in our task there was a fixed delay between water port poke and reward delivery. A neuron that modulates

firing rate during delay period is shown in Fig. 7. This neuron monotonically

Figure 7: Firing rate modulation during reward delay.



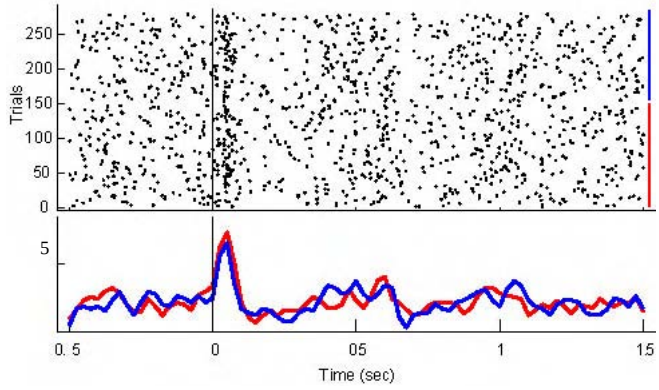
Top panel shows a raster plot of a non-5HT neuron locked to entry into the water port. *Bottom panel* shows PSTH of the same neuron. *Red* trace is the rewarded responses while *blue* trace are randomly interleaved, unrewarded correct responses. Note monotonic decrease in firing rate during reward delay. *Blue* dots in top trace denote time of reward delivery, *red* dots denote exit from water port.

decreased its firing rate until the expected time of reward and stayed inhibited during the time of reward delivery. However, it showed an anticipatory increase in firing rate before the water valve was turned off (again a fixed period of 0.5 s). Thus the neuron was inhibited at time of expected reward. Interestingly in case of randomly unrewarded correct trials, the neuron continued to be inhibited until the rat poked its nose out of the water port. Such delay related activity during reward period may be involved in temporal discounting computations. These responses can be further tested by changing distribution of reward delays.

Phasic activation by water valve sound:

It was somewhat surprising that 50% neurons showed phasic activation to sound

Figure 8: Phasic activation of putative 5HT neuron by sound of water valve.



Top panel shows a raster plot of a 5HT neuron locked to water valve onset. *Bottom panel* shows PSTH of the same neuron. *Red* and *blue* traces denote trials with the 2 odors. The neuron shows a sharp peak in activity immediately after water valve onset resulting in a loud click.

of opening water valve (Fig. 8). Latency of activation was ~ 20 ms. There have

been earlier reports of tone responsive raphe neurons [10]. There can be various explanations for these sound evoked responses. Raphe nucleus sends projections to all areas along the auditory pathway from cochlear nucleus to primary auditory cortex. Thus the phasic response could be purely sensory involved in auditory processing and thus conform to the motor hypothesis discussed earlier. Dorsal raphe projects heavily to the inferior colliculus (IC), especially to those layers that integrate auditory and non-auditory inputs like from the amygdala [11]. Thus, serotonin may modulate responses of IC neurons to these non-auditory inputs that may be involved in manifestation of defensive behaviors in response to aversive stimuli. This goes hand in hand with the proposed role of serotonin in anxiety and stress related behaviors. Thus, it will be interesting to see if activitation is purely auditory or if it is also modulated by other factors like salience, aversive conditioning or reward.

KEY RESEARCH ACCOMPLISHMENTS

- Developed and characterized rodent behavior in a novel two-alternative olfactory discrimination task and successfully adapted it to mice.
- Developed novel cannulated microdrive assembly for long term tetrode recordings in deep brainstem structures.
- Recorded neural activity of raphe neurons during goal directed behavior and found phasic modulation of firing rates correlated to specific behavioral events.

REPORTABLE OUTCOMES

1. Work toward the degree of Ph.D. for Sachin Ranade, Neurobiology and Behavior Program, Stony Brook University.
2. Obtained research support from the Thomas Hartman Foundation For Parkinson's Research.
3. Presented poster at Computational and Systems Neuroscience 2006 (CoSyNe 2006) in Salt Lake City, Utah.

CONCLUSIONS

The goal of this project was to record activity of serotonin and dopamine neurons during goal directed behavior. During the course of the project we have overcome a number of technical hurdles in order to successfully record neural activity of serotonergic and non-serotonergic neurons within the midbrain raphe nuclei of behaving rats. We found that contrary to earlier studies that reported mainly tonic long timescale changes in firing rates, raphe neurons phasically respond to task events on a short timescale of several milliseconds during various phases of the task spanning sensory, motor and reward related processes. We are encouraged by these results and are confident that this approach of recording from raphe neurons during goal directed behaviors will provide a new understanding of function of serotonin in aspects of cognition,

emotion and behavior. We now plan to investigate raphe neuronal responses during specific behaviors such as delayed reinforcement and reversal learning which are perturbed by serotonin dysfunction. We also plan to study the interaction between raphe neuronal firing and sniffing. Having established the technically challenging raphe recording technique we can now focus our attention on performing dual serotonin and dopamine neuron recordings in the context of reinforcement learning to fulfill the other goal of this project. Finally we want to complement our recording techniques with the immense potential afforded by mouse transgenic and knockout technologies and targeted gene delivery methods in order to move from correlations with behavior to a more mechanistic understanding by manipulating behavior.

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